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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/810,358	03/26/2004	Ker-Sang Chen	9188R&	1244

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THE PROCTER & GAMBLE COMPANY
Global Legal Department - IP
Sycamore Building - 4th Floor
299 East Sixth Street
CINCINNATI, OH 45202

EXAMINER	
SHAFER, SHULAMITH H	
ART UNIT	**PAPER NUMBER**
1647	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/810,358	CHEN ET AL.	
	Examiner	Art Unit	
	SHULAMITH H. SHAFER	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *23 November 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3,5-8,11-21 and 24-45* is/are pending in the application.

4a) Of the above claim(s) *13-15 and 24-45* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3,5-8,11,12,16-21* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Detailed Action

Status of Application, Amendments, And/Or Claims:

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 23 November 2009 has been entered.

The amendment received 23 November 2009 has been entered. Claims 22 and 23 have been canceled. Claim 1 has been amended and the amendment made of record.

Claims 1, 3, 5-8, 11-21, and 24-45 are pending in the instant application. Claims 13-15 and 24-45 stand withdrawn as being drawn to a non-elected invention.

Claims 1, 3, 5-8, 11, 12, and 16-21 are under consideration.

Withdrawn Rejections

Claims 22 and 23 have been canceled, thereby rendering all rejections of these claims moot.

The rejection of Claim(s) 1, 3, 5-8, 11, 12 and 16-21 under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement (**scope of enablement rejection**) is withdrawn in light of Applicants' amendment to the claims.

The rejection of Claims 1, 3, 5, 16, 17, 19, and 20 under 35 U.S.C. 103(a) as being unpatentable over Togawa et al. (2002 Am J. Physiol, Gastrointestinal Liver Physiol 283:G187-G195) is withdrawn in light of Applicants' amendment to the claims. New rejections are set forth below.

The rejection of Claims 18 and 21 under 35 U.S.C. 103(a) as being unpatentable over Togawa et al. as applied to claims 1, 17 and 20 in view of Vignali et al. is withdrawn in light of Applicants' amendment to the claims. New rejections are set forth below.

The rejection of Claims 6-8 under 35 U.S.C. 103(a) as being unpatentable over Togawa et al. as applied to claim 1 in view of Blumberg et al. (1999. Current Opinion in Immunology 11:648-656) is withdrawn in light of Applicants' amendment to the claims. New rejections are set forth below.

The rejection of Claims 11 and 12 under 35 U.S.C. 103(a) as being unpatentable over Togawa et al. as applied to claim 1 in view of Bing et al (1998. World J Gastroenterology 4:252-255) is withdrawn in light of Applicants' amendment to the claims. New rejections are set forth below.

Rejections

35 U.S.C. § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 1, 3, 5, 16, 17, 19 and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Togawa et al. (2002 Am J. Physiol, Gastrointestinal Liver Physiol 283:G187-G195) in view of Hart et al. (2003. J. Clin Gastroent 36:111-9)

Togawa et al. teach a method of determining the efficacy of lactoferrin treatment of animals with experimentally induced inflammatory bowel disease (abstract, page G187, 2nd column, last paragraph, G188, 1st column, 3rd paragraph). The reference evaluates the efficacy of treatment by comparing levels of anti-inflammatory cytokines and pro-inflammatory cytokines in TNBS-administered rats receiving lactoferrin (treated animals) to levels in control animals (TNBS-administered rats receiving 0.9% saline) in specimens of inflamed colon (biopsy sample of bowel) (page G188, 2nd column, last paragraph bridging page G189, 1st column, 1st paragraph and Figure 5). The anti-inflammatory cytokines measured were IL-4 and IL-10; the pro-inflammatory cytokines measured were TNF-α, IL-1β and IL-6 (abstract, page G-189, 1st column, 1st paragraph and Figure 5). Levels of cytokines were determined by ELISA assay (page G189, 1st column, 1st paragraph). The reference teaches that TNBS-induced colitis is a well-established model that is similar to human inflammatory bowel disease and teaches that activation of proinflammatory cytokines, such as TNF-α, IL-1β and IL-6, was suppressed by lactoferrin administration, while IL-4 and IL-10 in the colonic tissue was activated by lactoferrin, thereby contributing to the anti-inflammatory effect of lactoferrin (page G192, 2nd column, 2nd paragraph, bridging page G193, 1st column, 2nd paragraph), thus teaching a change in levels of pro-inflammatory and anti-inflammatory cytokines in response to lactoferrin treatment.

Togawa et al does not teach a method of determining the efficacy of a probiotic as a treatment of inflammatory diseases of the bowel in mammals. Hart et al. teach animal models and controlled clinical studies have demonstrated the

efficacy of probiotics in the maintenance of remission of ulcerative colitis and treatment of Crohn's disease (abstract, page 112, 2nd column, bridging page 115, 2nd column and Table 1). Thus, it would have been obvious to the person of ordinary skill in the art at the time the invention was made to substitute a method of determining the efficacy of a probiotic treatment of inflammatory diseases of the bowel in mammals, as taught by Hart et al for a method of determining the efficacy of lactoferrin treatment of inflammatory diseases of the bowel in mammals. One would be motivated to make such a substitution and anticipate success because both lactoferrin and probiotics are taught in the art as effective treatments for inflammatory diseases of the bowel and monitoring efficacy of treatment by determining levels of pro- and anti-inflammatory cytokines is taught by Togawa et al.

Additionally, neither reference, singly or in combination, directly teaches measuring the level of at least one anti-inflammatory cytokine and at least one pro-inflammatory cytokine before treatment and determining the ratio of the level of the at least one anti-inflammatory cytokine to the level of the at least one pro-inflammatory cytokine before treatment and measuring the level of at least one anti-inflammatory cytokine and at least one pro-inflammatory cytokine after treatment and determining the ratio of the level of the at least one anti-inflammatory cytokine to the level of the at least one pro-inflammatory cytokine after treatment.

However, it would have been obvious to the person of ordinary skill in the art at the time the invention was made, treating IBS patients, to measure cytokine levels in a biological sample before administration of treatment (equivalent to measuring cytokine levels in control, untreated animals with TNBS-induced colitis) and after treatment to assess efficacy of treatment. A person of ordinary skill in the art would have been motivated to make those modifications because Togawa et al teach administration of lactoferrin as a potentially attractive therapeutic strategy for the treatment of inflammatory bowel disease and Hart et al teach administration of probiotics as a potentially attractive therapeutic

strategy for the treatment of inflammatory bowel disease thus suggesting clinical experimentation to determine efficacy of the administration of lactoferrin or probiotics, as treatment of bowel disease. The skilled artisan, following the teaching of Togawa et al, would be motivated to measure cytokine levels before treatment in a clinical setting, instead of measuring levels in control animals, as the artisan would be aware of that such study design is standard protocol in clinical research. Furthermore, knowing the results of measurements of cytokine levels (as shown, for example, in Togawa et al, Figure 5), one would be motivated to compute ratios as a convenient way of determining shifts in patterns of cytokine levels. One would reasonably expect success because methods of measuring cytokine levels in biological samples is well known in the art, and is taught by Togawa et al.

Applicants traverse the rejection as previously presented (Response of 23 November 2009, page 11, 4th paragraph, bridging page 12, 1st paragraph). The reasons for the traversal are:

(a) Claim 1 is amended and now directed to methods of determining the efficacy of a probiotic as a treatment of inflammatory diseases of the bowel in mammals. Towaga teaches administration of TNBS to induce colitis and studies the use of lactoferrin to attenuate the induced colitis. As such, the rejection is now moot since fails to teach or suggest methods to determine the efficacy of probiotics in inflammatory diseases of the bowel.

(b) Towaga does not teach measuring cytokine levels before and after treatment *or* determining the ratios of cytokines before and after treatment. Towaga details only one particular experiment in rats using induced colitis. Towaga does not provide any suggestion or motivation to study 'before and after' results, or to set up such experiments, without controls. Towaga does not suggest studying ratios of cytokines to establish and analyze shifts in patterns of cytokine levels to evaluate efficacy of treatment and does not suggest or provide motivation for the particular cytokines and ratios as set forth in the claims.

Applicant's arguments have been fully considered but are not found to be persuasive for the following reasons:

In response to (a): In view of Applicants' amendment of the claims, the rejection has been recast to include Hart et al., which teaches the efficacy of probiotics in treatment of inflammatory bowel disease.

In response to (b): One of ordinary skill in the art, aware of art-accepted protocols in clinical research, would be aware that measurement of cytokine levels in diseased animals treated with saline (control animals) would be the experimental equivalent of measurement of cytokine levels before beginning treatment in a clinical setting. Togawa et al. suggests extending the finding of the treatment study to a clinical setting, while Hart et al. teaches administration of probiotics to treatment inflammatory bowel disease in humans. Togawa et al teaches measuring levels of recited anti-inflammatory cytokines, IL-4 and IL-10 and measuring levels of recited pro-inflammatory cytokine, TNF-α in control and experimentally treated animals, the equivalent of measuring levels before and after administration of treatment. Once levels of pro- and anti-inflammatory cytokines are determined, one would be motivated to compute ratios of anti-inflammatory to pro-inflammatory cytokines as an easy way of determining shifts in patterns of cytokine levels.

Claims 18 and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Togawa et al. and Hart et al. as applied to claims 1, 17 and 20 in view of Vignali et al. (cited in previous Office Action). The teachings of Togawa et al. and Hart et al are outlined above. The references, singly or in combination, do not teach a method of measuring levels of at least one anti-inflammatory cytokine and at least one pro-inflammatory cytokine in a biological sample by multiplexed ELISAs using coded microspheres coupled with a flow cytometer detection system. Vignali et al teach a FlowMetrix System of quantifying the concentration of 15 cytokines simultaneously in a 100 µl sample.

The Luminex FlowMetrix system uses microspheres as the solid support for a conventional immunosorbent assay. Each bead set is comprised of microspheres manufactured with a uniform, distinct proportion of red and orange fluorescent dyes. Data are acquired on a conventional flow cytometer (page 246, 1st column, 1st paragraph).

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the teachings of Togawa et al and Hart et al which teach evaluating the efficacy of a probiotic treatment by measuring cytokine levels using an ELISA assay and substitute the multiplex assay taught by Vignali for the ELISA assay taught by Togawa et al. The person of ordinary skill in the art would have been motivated to make these modifications and anticipate success because Vignali teaches the multiplex system simultaneously measures many different analytes in a small sample volume (abstract) measuring a number of cytokines simultaneously in biological fluids and tissue culture samples.

Applicants traverse the rejection as previously presented (Response of 23 November 2009, page 12, 3rd paragraph bridging page 13, 2nd paragraph). The reasons for the traversal are:

(a) Claim 1 is directed to methods of determining the efficacy of a probiotic as a treatment of inflammatory diseases of the bowel in mammals. Claims 18 and 21 are dependent upon Claim 1 and are therefore affected by this amendment. Towaga teaches administration of TNBS to induce colitis and studies the use of lactoferrin to attenuate the induced colitis. As such, the rejection is now moot since fails to teach or suggest methods to determine the efficacy of probiotics in inflammatory diseases of the bowel.

(b) One of ordinary skill in the art would not have been motivated to make the jump from one type of rat study to a different type of human study, simply because clinical study techniques are generally known, even if Towaga were combined together with Vignali and a FlowMetrix ™ system were used. Simply because Vignali discloses a type of assay useful for measuring cytokines,

and Towaga discloses a particular rat experiment in which cytokines were measured, does not disclose or suggest, or provide motivation or expectation of success for determining particular cytokines to measure and compare in humans, to use as a way to test and evaluate efficacy of treatments for IBS in humans. One would not have arrived at the claimed method of determining the efficacy of a treatment of inflammatory diseases of the bowel in mammals *in vivo.* Towaga and Vignali together do not suggest or provide motivation or expectation of success for a clinical method, using samples from a biological subject, in which particular cytokine levels are determined and ratios are analyzed.

Applicant's arguments have been fully considered but are not found to be persuasive for the following reasons:

In response to (a): In view of Applicants' amendment of the claims, the rejection has been recast to include Hart et al., which teaches the efficacy of probiotics in treatment of inflammatory bowel disease.

In response to (b): In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., evaluation of efficacy of treatment in humans) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

Togawa et al suggest building upon his findings and undertaking clinical experimentation to determine efficacy of the administration of lactoferrin, while Hart et al teaches the efficacy of probiotics in the treatment of inflammatory bowel disease. One is always motivated to treat diseases in a clinical setting. The clinical researcher would be motivated to measure cytokine levels before treatment in a clinical setting, instead of measuring levels in control animals as taught by Togawa et al, since such is standard practice in clinical research. Togawa et al teach measuring levels of at least one anti-inflammatory cytokine, wherein the cytokine is IL-4 and/or IL-10 and at least one pro-inflammatory

cytokine wherein the cytokine is TNF-α (as recited in the claims of the instant invention) in a biological sample by ELISA. It would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the teachings of Togawa et al and Hart et al. and substitute the multiplex assay taught by Vignali for the ELISA assay taught by Togawa et al. One would be motivated to make this substitution, and anticipate success since both assays involve immunological methods of measuring cytokine concentrations and Vignali teaches a more efficient method of quantifying the concentration of 15 cytokines simultaneously. As stated above, knowing the results of measurements of cytokine levels, one would be motivated to compute ratios as a way of determining shifts in patterns of cytokine levels.

Claims 6-8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Togawa et al. and Hart et al as applied to claim 1 in view of Blumberg et al. (1999. Current Opinion in Immunology 11:648-656). The teachings of Togawa et al. and Hart et al are outlined above. Togawa et al do not teach method wherein the ratio of anti-inflammatory cytokine to pro-inflammatory cytokine is the ratio of the level of IL-10/to the level of IL-12 (claim 6), or the level of TGF-β/to the level of IL-12 (claim 7), or the level of IL-10/ to the level of IFN-γ (claim 8). Togawa et al. teaches measurement of anti-inflammatory cytokines IL-4 and IL-10 and measurement of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 (abstract and Figure 5). Blumberg et al. teach immune responses uniquely involved in IBD pathogenesis and note the importance of balance of pro-inflammatory cytokines such as IFN-γ, TNF, and IL-12 and anti-inflammatory cytokines such as IL-10 and TGF-β (abstract). The reference teaches that IL-12 is a key factor in the pathogenesis of the TNBS-induced colitis model (the model taught by Togawa et al) and induces overproduction of IFN-γ and TNF (page 650, 2nd column, last paragraph bridging page 651, 1st column, 3rd paragraph). Blumberg et al also teach that mucosal inflammation can be viewed as a failure of production of

suppressor cytokines such as TGF-β and IL-10 (page 652, 2nd column, 2nd paragraph).

Aware of the teachings of Blumberg, which identify pro- and anti-inflammatory cytokines crucial to the pathology of IBD, it would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the teachings of Togawa et al and substitute measurement of the pro-inflammatory cytokines taught by Blumberg et al (IFN-γ and IL-12) for the pro-inflammatory cytokine taught by Togawa et al (TNF-α, IL-1β and IL-6) and the anti-inflammatory cytokine taught by Blumberg et al (TGF-β) for the anti-inflammatory cytokine taught by Togawa et al (IL-10). Once measurement of these cytokines is accomplished, the calculation of ratios would be obvious as a way of monitoring changes in the balance of levels of pro- to anti- inflammatory cytokines. One would be motivated to make these modifications because both references teach the importance of disturbed balance between proinflammatory and anti-inflammatory cytokines in the pathology of inflammatory bowel disease and Blumberg et al teach IFN-γ, TNF, and IL-12 are pro-inflammatory cytokines involved in pathology of IBD (art-recognized equivalents) and IL-10 and TGF-β are anti-inflammatory cytokines (art-recognized equivalents) whose expression may be down-regulated in IBD. One would have expected success because methods of measuring cytokine levels in biological samples is well known in the art, and is taught by Togawa et al.

Applicants traverse the rejection as previously presented (Response of 23 November 2009, page 13, 4th paragraph bridging page 14, 3rd paragraph). The reasons for the traversal are:

(a) Claim 1 is directed to methods of determining the efficacy of a probiotic as a treatment of inflammatory diseases of the bowel in mammals. Claims 6 – 8 are dependent upon Claim 1 and are therefore affected by this amendment. Towaga teaches administration of TNBS to induce colitis and studies the use of lactoferrin to attenuate the induced colitis. As such, the

rejection is now moot since fails to teach or suggest methods to determine the efficacy of probiotics in inflammatory diseases of the bowel.

(b) Towaga does not suggest establishing or analyzing any ratios of cytokines, nor particularly the claimed ratios. Blumberg also does not suggest establishing or analyzing ratios of cytokines, nor the importance or utility thereof for testing or determining efficacy of a potential treatment. Blumberg simply notes that there is likely an on-going balance between pro- and anti-inflammatory cytokines, and their release and activity in body systems in relation to inflammation. Blumberg does not suggest or provide motivation, expectation of success or predictability for the particular claimed methods of evaluating efficacy of treatments. Simply because levels of various cytokines can be measured and various experiments can be run in animal models does not provide the requisite motivation or expectation of success for selecting and measuring particular cytokines and monitoring ratios thereof, in humans, for screening and evaluating the efficacy of a potential treatment. Neither Towaga nor Blumberg provide motivation for methods of screening compositions for efficacy in treating diseases of the bowel in humans.

Applicant's arguments have been fully considered but are not found to be persuasive for the following reasons:

In response to (a): In view of Applicants' amendment of the claims, the rejection has been recast to include Hart et al., which teaches the efficacy of probiotics in treatment of inflammatory bowel disease.

In response to (b): In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., evaluation of efficacy of treatment in humans) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

While Towaga et al and Blumberg do not teach or suggest establishing or analyzing any ratios of cytokines to evaluate efficacy of treatments, once levels of cytokines are determined, the calculation of ratios would be obvious as a way of monitoring changes in the balance of levels of pro- to anti- inflammatory cytokines. Towaga teaches measurement of levels of anti-inflammatory cytokines IL-4 and IL-10 and measurement of pro-inflammatory cytokines TNF-α, IL-1β and IL-6. The teachings of Blumberg et al are directed to inflammatory bowel disease, and note the importance of a balance of pro-inflammatory cytokines such as IFN-γ, TNF, and IL-12 and anti-inflammatory cytokines such as IL-10 and TGF-β. Thus, one of ordinary skill would recognize that anti-inflammatory cytokines such as IL-4 (taught by Towaga), IL-10 (taught by both Towaga and Blumberg) and TGF-β (taught by Blumberg) are art-recognized equivalents and pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 (taught by Towaga) and IFN-γ, TNF, and IL-12 (taught by Blumberg) are art-recognized equivalents. The measurement of levels of any one of the specifically listed anti- and pro-inflammatory cytokines before and after treatment would provide equivalent information about the efficacy of the administered treatment. As stated above, once levels of cytokines are determined, the calculation of ratios would be obvious as a way of monitoring changes in the balance of levels of pro- to anti- inflammatory cytokines. Therefore, a method of measuring cytokine levels and calculating levels of anti- to pro-inflammatory cytokines wherein the ratio of anti-inflammatory cytokine to pro-inflammatory cytokine is the ratio of the level of IL-10/to the level of IL-12, or the level of TGF-β/to the level of IL-12, or the level of IL-10/ to the level of IFN-γ is obvious over the teachings of the prior art.

Claims 11 and 12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Togawa et al. and Hart et al. as applied to claim 1 in view of Bing et al (1998. World J Gastroenterology 4:252-255, cited in previous Office Action).

The teachings of Togawa et al. and Hart et al. are outlined in detail above. Togawa et al does not teach a method of determining the efficacy of a treatment of inflammatory disease of the bowel in mammals wherein said biological sample comprises peripheral blood mononuclear cells (PBMC) with *in vitro* stimulation (Claim 11), wherein said in vitro stimulation comprises stimulation with a mitogen (Claim 12).

Bing et al. teach assaying production of inflammatory cytokines such as TNF-α and IL-6 by PBMCs isolated from patients with IBS wherein said PBMCs are stimulated by a mitogen, PHA (phytohemagglutinin), thus teaching measurement of cytokine levels produced by PBMCs isolated from patients with IBS to be an equivalent method of determining cytokine profiles as measuring cytokine levels in biopsy samples from the bowel of IBS patients.

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the teachings of Togawa et al and Hart et al. and substitute measurement of the pro-inflammatory cytokines and anti-inflammatory cytokines in mitogen-stimulated PBMCs, the system taught by Bing et al, for measurement of cytokines in colonic tissue (biopsies) from control and treated animals (equivalent of “before" and “after” measurements of cytokine levels in human patients). One of ordinary skill in the art would have been motivated to make these modifications because the skilled artisan would recognize that it would be simpler and less invasive to obtain PBMCs from blood samples drawn from patients than to obtain biopsies from colon tissue. Once measurement of these cytokines is accomplished, the calculation of ratios would be obvious as a way of monitoring changes in the balance of levels of pro- to anti- inflammatory cytokines. One would have expected success because methods of measuring cytokine levels in cell culture supernatants is well known in the art, and is taught by Bing et al.

Applicants traverse the rejection as previously presented (Response of 23 November 2009, page 14, last paragraph bridging page 15, 3rd paragraph). The reasons for the traversal are:

(a) Claim 1 is directed to methods of determining the efficacy of a probiotic as a treatment of inflammatory diseases of the bowel in mammals. Claims 11 and 12 are dependent upon Claim 1 and are therefore affected by this amendment. Towaga teaches administration of TNBS to induce colitis and studies the use of lactoferrin to attenuate the induced colitis. As such, the rejection is now moot since fails to teach or suggest methods to determine the efficacy of probiotics in inflammatory diseases of the bowel.

(b Towaga does not suggest establishing or analyzing any ratios of cytokines, nor particularly the claimed ratios, and that one of skill in the art would not have been led by Towaga's rat study to perform a completely different human study. Bing studied stimulated release of various cytokines by PMBCs in patients with UC (ulcerative colitis) compared to healthy controls, and suggested possible reasons for the results, including active disease state, genetic heritage, and medication. Bing looked for correlation between TNF-alpha, IL-6 and sIL-2r production and disease activity, disease location and medication. However, Bing does not suggest or provide motivation, expectation of success or predictability for the particular claimed methods of measuring particular cytokines, measuring cytokine levels in the same subject (versus subjects and controls as in Bing) before and after treatment, and using particular ratios of cytokines in methods of evaluating efficacy of potential treatments. Neither Towaga nor Bing provide the requisite motivation for the Applicants' particular methods. Simply because one *can* measure cytokines, cytokine levels have been measured in various studies, and one *could* in theory calculate various ratios of cytokines, would not have led one of skill in the art to the present invention. The cited documents do not suggest using particular cytokines to screen potential treatments for inflammatory diseases of the bowel, nor provide motivation or expectation of

success for developing a screening method for evaluating potential treatments for inflammatory diseases of the bowel.

Applicant's arguments have been fully considered but are not found to be persuasive for the following reasons:

In response to (a): In view of Applicants' amendment of the claims, the rejection has been recast to include Hart et al., which teaches the efficacy of probiotics in treatment of inflammatory bowel disease.

In response to (b): In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., evaluation of efficacy of treatment in humans) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

As discussed above, Togawa et al measure levels of a pro- and anti-inflammatory cytokine recited in the methods of the claimed invention, IL-10 and IL-4. Towaga teaches the measurement of levels of said cytokines in control and treated animals with TNBS-induced inflammatory bowel disease (the equivalent of "before" and "after" measurements to establish the efficacy of treatment in clinical studies). The Bing reference is provided to establish that cytokines can be measured in supernatants of stimulated PBMCs as an alternative and less invasive method for evaluating changes in cytokine levels in mammalian subjects (stimulated PBMCs vs biopsies). Computing ratios of levels of anti-inflammatory cytokine to pro-inflammatory cytokine is a mental exercise or calculation step and would not confer patentability on the method of the instant invention. Once measurement of the recited cytokines is accomplished, the calculation of ratios would be obvious to the skilled artisan as a way of monitoring changes in the balance of levels of pro- to anti- inflammatory cytokines

Conclusions:

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SHULAMITH H. SHAFER whose telephone number is (571)272-3332. The examiner can normally be reached on Monday through Friday, 8 AM to 5 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Nickol, Ph.D. can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Shulamith H. Shafer/
Examiner, Art Unit 1647